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(54) Monitoring or diagnosing fatigue related illness

(57) Body phosphate stores are measured to monitor or diagnose fatigue related illness such as myalgic encephalomyelitis (ME) or post viral fatigue syndrome (PVFS). Phosphate is typically AMP, ADP or ATP and may be measured by an enzyme based assay using luciferase and test samples of urine or plasma where the concentration of K, Ca or Mg ions is further measured. Further claimed is the use of phosphate in the manufacture of a medicament for treating fatigue related illness.

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

	Create	C	K	C	Mg	C	P04	C	Ca	C	10-65 PTH	C	TmP/ GFR	C	Urine Creat	Urine P04
R1	107	78	4.2	3.8	0.85	0.83	1.04	0.93	2.3	2.36	21	44	0.86	1.01	4.1 11.7	6.9 9.2
M2	84	70	3.9	3.9	0.94	0.94	0.74	1.12	2.3	2.45	44	31	0.66	0.96	6.8 9.2	7.1 21.2
C3	56	74	3.7	3.6	0.85	0.86	1.02	1.27	2.33	2.34	13	21	0.88	1.14	1.4 8.4	3.6 17.2
H4	79	68	3.8	3.7	0.85	0.73	1.00	0.71	2.34	2.33	22	22	0.68	0.92	3.7 3.1	15.2 1.0
P5	72	80	4.1	4.3	0.88	0.85	0.80	1.72	2.52	2.35	31	35	0.68	1.80	2.8 12.0	4.1 20.3
A6	80	70	3.4	4.1	0.85	0.73	1.03	1.00	2.38	2.29	24	32	0.86	1.16	5.1 1.0	10.7 0.8
J7	118	80	3.6	3.8	0.81	0.80	0.67	1.26	2.43	2.38	31	32	0.56	1.06	3.0 11.5	2.9 28.0
Z8	84	84	3.7	5.0	0.87	1.06	0.73	0.80	2.36	2.35	60	28	0.54	0.74	11.9 3.5	27.1 3.7
M9	84	86	4.0	3.9	0.80	0.90	0.83	1.48	2.32	2.47	24	18	0.86	1.28	1.7 9.2	1.4 20.2
P10	73	83	3.6	4.4	0.70	0.81	0.89	0.99	2.31	2.30	36	27	0.99	1.21	14.1 15.2	11.4 7.8

All results save PTH. TmP/GFR, urine creatinine are quoted in m mol/l, PTH ng/l, TmP/GFR urine creatinine μmol/l

Table of plasma concentrations of ten 'ME' sufferers matched with ten age and sex matched controls.

Creat + Creatinine K = Potassium Mg = Magnesium P04 = Phosphate PTH = Parthyroid hormone
TmP/GFR = Tubular maximum of phosphate reabsorption/Glomerular filtration rate C = Control result

FATIGUE RELATED DISEASES

The invention relates to a method for diagnosing and monitoring a disease, hitherto of controversial status and variously named but commonly known as chronic fatigue syndrome or myalgic encephalomyelitis (ME) or post viral fatigue syndrome (PVFS); test kits for carrying out said diagnosis and monitoring; and treatments for said disease.

ME or PVFS is the general term given to a chronic fatigue syndrome in the United Kingdom,
Australia and New Zealand. It may correspond to la spasmophilie in France and it is known in the United States of America and Canada as chronic fatigue/immune dysfunction syndrome. Moreover, chronic fatigue syndrome overlaps with the term fibromyalgia. In Japan it is known as the "low natural killer cell syndrome" and it is of note that similar diseases have been described in animals and in particular in race horses.

The controversial nature of the disease stems, in part, from the fact that there is no definitive means of diagnosis, rather there exists a triad of symptoms which form the "London Criteria" for the diagnosis of ME/PVFS for research purposes exhibited by individuals thought to have ME/PVFS. These symptoms include:

(a) fatiguability after mental or physical exercise with slow recovery;

(b) cognitive disturbance, short term memory, neurogenic pain, disturbance of other body functions; and

(c) fluctuations of symptoms from hour to hour and day to day.

In addition, this lack of definitive diagnosis has resulted in a lack of terminology for the disease, hence the different terms used throughout the world to describe a disease characterised by the above symptoms, and also even a reluctance to accept and/or acknowledge the disease.

The most distinctive symptom in this type of disease is fatiguability of muscle and brain following either sustained or repetitive use with delayed and slow recovery. In some patients there may be a delay of twenty four to forty eight hours before the onset of fatigue and more general symptoms are noticed. In many there is a predictable pattern and time course for recovery. Ergometric studies may show no lack of power, but a subject's inability to continue a task has been interpreted variously as of central origin or due to unwillingness rather than physiological dysfunction. This latter interpretation has been made even though early transfer to an anaerobic condition and lactic acid production has been shown to occur.

We began our investigations by postulating that the basis for the fatiguability is a defect in the regeneration of high energy phosphates, ATP, ADP, AMP, and 2,3 DPG, in the basic energy cycle so that restoration of normal energy levels may be delayed or even incomplete. We also considered that incomplete restoration, if allowed to continue, may result in damage to cell structures such as mitochondria and possible alterations to membrane based active transport. Such incomplete restoration may be secondary to intracellular ion depletion, ie one or more of the following; phosphate, potassium or magnesium. Of these we feel that phosphate may be the most crucial. This may result in a deceptively high or even normal plasma phosphate concentration when in fact over time there is a body phosphate depletion. When the aforementioned cell membrane damage happens in the kidney, there may be a constant drain of intracellular constituants into the urine.

However, changes in body phosphate stores would need to be very gross to be detected in routine biochemical assays because of the large body pool and powerful homoeostasis of phosphate, even though prolongation of a phosphate imbalance would lead to interference with every other bodily function dependent on ATP. We therefore speculated that the effect on renal tubular function would be a sensitive indicator of ATP depletion since this depletion will lead to a paradoxical failure to retain phosphate and thus reflect an unsustainable additional loss of phosphate in circumstances where there is a need to conserve phosphate. The end result would be a total body depletion of phosphate with interference in varying degrees with every other function such as immune reactions, cytokine production, hepatic detoxification of drugs and metabolites, gut mobility, neurotransmitter function, red cell shape and tissue respiration.

We also speculated that bioavailable high energy phosphate stores could be measured by extracting a sample of fluid, ideally blood, from an individual or animal to be tested and then performing an analysis and ideally an enzyme based analysis such as a luminescence assay based on firefly luciferin. This is thus a measure of energy charge.

Energy charge is defined as (ATP + 0.5ADP)/(ATP+ADP+AMP) luciferase.

The causative nature of the disease will not be addressed herein suffice to say that the nature of the disease gives rise to metabolic crisis of a chronic nature.

Our observations of individuals suffering from such fatigue related disease has enabled us to invent methods which for the first time enables, either direct or indirect reliable diagnosis of the disease and provides a scientific basis for therapy.

It is therefore an object of the invention to provide a method and means for diagnosing and monitoring the course of fatigue related diseases especially ME/PVFS and also to provide therapeutic treatments which reverse or at least mitigate the effects of fatigue related disease such as ME/PVFS.

In its broadest aspect the invention therefore concerns a measure of body phosphate stores with a view to diagnosing and monitoring a fatigue syndrome and more preferably a chronic fatigue syndrome.

The following is an example of what can be termed an indirect method of analysis in so far as it enables us to extrapolate the data obtained by the method in order to determine a picture of body phosphate levels.

According to a first aspect of the invention there is therefore provided a method for diagnosing and monitoring fatigue related illnesses comprising taking a plasma and a urine sample from an individual or animal to be tested; measuring the phosphate concentration in said plasma and urine; and measuring the concentration of at least one other ion in the plasma which ion is either potassium, magnesium or calcium whereby this information can be used to determine the renal phosphate clearance/reabsorption and the plasma concentration of said at least one pre-selected ion so as to compare said values with that of a normal individual or animal.

In the diagnosis of the invention we have discovered that individuals with no primary renal disorder show a marked reduction in renal phosphate clearance/reabsorption and this is also paralleled with a marked reduction in the plasma concentration of said one pre-selected ion. We can therefore reliably and repeatedly use this information for diagnosing and monitoring fatigue related diseases especially ME/PVFS.

In a preferred diagnosis of the invention renal phosphate clearance/reabsorption is determined using the TmP/GFR ratio (a measure of the renal tubular reabsorption of phosphate).

The following is an example of what can be termed a direct method for determining bioavailable high energy phosphate in that it enables a determination of either red blood cell or leucocyte ATP, ADP and AMP levels.

According to a further aspect of the invention there is therefore provided a method for diagnosing and monitoring fatigue related illnesses comprising taking a leucocyte or red blood cell sample from an individual or animal to be tested and measuring the high energy phosphate stores in the form of energy charge in said sample.

In this further aspect of the invention a technique is used which is an enzyme based technique which ideally employs the use of an enzyme which is phosphate dependent, that is to say an enzyme that functions by the use of high energy phosphate and therefore the activity of the enzyme can be used as a direct measurement of the amount of high energy phosphate stores.

More preferably, the enzyme catalyses, either directly or indirectly a reaction involving light emittance and the use of bioavailable high energy phosphate stores such as, for example, the enzyme luciferase.

Using our methods of diagnosis we have found that individuals suffering from fatigue related diseases exhibit hypophosphataemia [by this we mean a reduction in total body phosphate, rather than merely a reduction in plasma phosphate concentration] due to phosphate depletion in the absence of redistribution. It

follows that the diagnosis of the disease also helps to characterise it and this characterisation can then be extrapolated to a method of treatment. For example, the hypophosphataemia which we have identified during the diagnosis of the disease may be alleviated and/or reversed by the administration of phosphate.

According to a third aspect of the invention there is therefore provided the use of phosphate in the manufacture of a medicament for treatment of a fatigue related disease.

Preferably said fatigue related disease is ME/PVFS.

In a preferred embodiment of the invention said medicament is of an infusible nature.

In yet a further preferred embodiment of the invention said medicament is of an oral nature such as an oral phosphate supplement.

According to a yet fourth aspect of the invention there is provided a test kit for diagnosing and monitoring a fatigue related disease comprising a means for determining renal phosphate clearance/reabsorption and a means for determining the plasma concentration of at least one pre-selected ion.

Preferably said fatigue related disease is ME/PVFS.

In a preferred embodiment of the invention said kit further comprises means for taking a blood and/or urine sample and in the former instance means for providing a plasma extract of said blood sample.

In yet a preferred embodiment of the invention said means for determining the plasma concentration of a pre-selected ion includes means for determining the plasma concentration of either potassium, and/or magnesium and/or calcium.

According to a yet further aspect of the invention there is provided a test kit for diagnosing and monitoring a fatigue related disease comprising a means for determining bioavailable high energy phosphate stores in a sample.

In a preferred embodiment of the invention said means comprises at least an enzyme and more preferably an enzyme that is phosphate dependent such as for example luciferase.

The method of the invention is, as aforesaid, useful for diagnostic purposes to determine whether an individual is suffering from a fatigue related disease. In addition, the method is also useful in that it can be used generally to reflect the clinical state of a patient, it can indicate intracellular ion depletion and it can reflect the intracellular phosphate stores of an individual.

In addition, the invention also has utility in that:

- the fatigue related disease ME/PVFS can now be defined in biochemical terms;
- ME/PVFS may be distinguished from other types of fatigue;
- the diagnosis will prevent much pain and suffering from undiagnosed or misdiagnosed illness;

- the diagnosis will direct appropriate therapy and save costs from unnecessary and futile investigation and inappropriate treatment;

- the diagnosis can be applied at any stage of the illness, at the beginning or years after onset;

- the diagnosis can be applied to conditions affecting energy metabolism which could previously only be investigated by muscle biopsy and elaborate and expensive indirect methods;

- the same diagnosis can be used to monitor therapy and spontaneous fluctuations of health;

- modifications of the diagnosis by repeating it after exercise can be applied to the study of other disease states in which fatigue is a problem, which can be distinguished by the presence of additional symptoms, or the results of laboratory and/or other investigations;

- the diagnosis will be invaluable to insurance and benefit agencies and litigation about causation and nature of illness;

- the diagnosis will allow proper epidemiological and prevalence studies to be undertaken, and comparisons to be made with immunology and virological studies;

- the diagnosis can be applied to animals with appropriate adjustments;

- the diagnosis, taken in conjunction with a direct measurement of energy charge defined as (ATP + 0.5 ADP)/(ATP + ADP + AMP), gives a complete picture of the energy metabolism of the patient;

- improved treatment based on the diagnosis will allow many more individuals to return to health and employment.

Embodiments of the invention will now be described, by way of example only, with reference to the single table and following methods, results and treatments.

Performing the Diagnosis/Monitoring the Disease

The method comprises taking a sample of blood under anaerobic conditions and mixing said sample with an anticoagulant. This is followed by immediate separation of the plasma from cells in order to prevent the diffusion of ions.

A sample of urine collected from the individual at the same time is also taken to be tested.

Thereafter, using conventional techniques, an estimation of the plasma and urinary phosphate concentrations are made. From this information, using conventional calculations renal phosphate clearance can be determined. Typically, the renal phosphate clearance is determined by the TmP/GFR ratio which is derived from Bijvoet's nomogram.

In addition, the plasma level of at least one pre-selected ion such as potassium, magnesium or calcium is also determined using conventional techniques.

Test Results

The accompanying table represents the results obtained when the above test was performed on ten individuals which were thought to be suffering from fatigue related diseases. Examining the table from left to right, a measurement was made of the plasma concentration of creatinine and then plasma concentrations of potassium, magnesium, phosphate, molecular calcium and ionic calcium. In addition, the TmP/GFR ration was also determined. C represents those figures obtained from the control, that is an aged and sexed-matched individual deemed to be normal.

We discovered that the TmP/GFR ratio demonstrates a statistically highly significant difference from paired controls and when coupled with abnormalities in the concentration of one or more plasma ion, we had identified a characterising feature of the disease and thus a means for diagnosing same. For example, individuals showing the triad of symptoms hereinbefore described and also suspected to be suffering from ME/PVFS such as the individual referred to as "J", showed a marked difference from age and sex-matched control in TmP/GFR ratio and also a marked difference in the concentration of plasma potassium and phosphate ions. Similarly, the individual referred to as "M" also showed a marked difference in TmP/GFR along with a marked difference in plasma phosphate concentrations. These results are indicative of intracellular ion depletion which we believe is the underlying cause of many of the symptoms of this disease.

Alternative Method for Performing the Diagnosis/Monitoring of the Disease

The method comprises taking a 2ml sample of heparinised blood from an individual or an animal to be tested and then lysing said sample within 10 minutes with 10% trichloroacetic acid. The sample is then centrifuged and the supernatant

is collected. The sample is diluted so that a final dilution of 1/50 is obtained and 10μl sample of this is used for analysis.

Analysis is carried out on a 125I luminometer from Bio Orbit (Turku, Finland) comprising a temperature controlled sample carousel for 25 cuvettes, and with three dispensers connected. The dispensers dispense:

1) ATP monitoring reagent (firefly luciferase) a 100μl
2) Pyruvate Kinase/phosphoenol pyruvate 10μl PEP-EDTA buffer containing Pyruvate Kinase (20 units per ml); and
3) Myokinase sequentially 10μl (150 units per ml).

A fourth dispenser dispenses ATP as standard. Measurement is an end point measurement of luminescence.

The advantage of this method is that firefly luciferin luciferase produces an almost constant light intensity proportional to ATP concentration.

Using the above dispensing protocol.

1) Measures ATP content
2) Measures ATP content from ADP; and
3) Measures ATP content from AMP and ADP.

Treatments for the Disease

We have established that the chronic hypophosphataemia in the absence of redistribution which we have discovered underlies the disease can be treated using phosphate supplements. We prefer to use a method according to Vannatta et al

(Vannatta JB, Whang R, Papper S. Efficacy of intravenous phosphate therapy in the severely hypophosphataemic patient. *Arch Intern Med* 1981; 141:885-87) which involves a maximum of 9 mmol/1 monobasic potassium hydrogen phosphate given over 12 hours for the treatment of severe hypophosphataemia (less than 0.32 mmol/l). However, even with this protocol we have found that plasma ionised calcium may fall to very low concentrations. This can happen even though patients may remain symptom free. However, the potential for severe hypocalcaemia is evident and we therefore adhered to the Vannatta regime while measuring plasma calcium, phosphate and potassium concentrations every 6 hours. In this way, problems associated with deficiencies in plasma calcium, phosphate and potassium can be identified and corrected. In addition, a measurement of renal output would be an early indicator of metastatic calcification of tissues.

CLAIMS

1. A method for monitoring fatigue related illnesses comprising measuring, either indirectly or directly, body phosphate stores.

2. A method according to Claim 1 wherein said indirect measurement comprises taking a plasma and a urine sample from an individual or animal to be tested; measuring the phosphate concentration in said plasma and urine; measuring the concentration of at least one other ion in the plasma, which ion is either potassium magnesium or calcium, whereby this information can be used to determine the renal phosphate clearance/reabsorption and the plasma concentration of said at least one preselected ion so as to compare said values with that of a normal individual or animal.

3. A method according to Claim 1 where said direct method comprises taking a leucocyte or red blood sample from an individual to be tested and measuring high energy phosphate stores in the form of the energy charge in said sample.

4. A method according to Claim 3 wherein said measurement of high energy phosphate store involves the use of an enzyme-based assay wherein the enzyme either directly or indirectly makes use of said phosphate so that use of said phosphate can be observed by observing the catalytic activity of the enzyme.

5. A method according to Claim 4 wherein said enzyme is luciferase.

6. A test kit for diagnosing and monitoring fatigue related illnesses comprising a means for measuring, either indirectly or directly, body phosphate stores

means for determining renal phosphate clearance/reabsorption and a means for determining the plasma concentration of at least one preselected ion.

8. A test kit according to Claim 7 wherein said ion is either potassium magnesium or calcium.

9. A test kit according to Claim 6 wherein said direct means comprises an enzyme assay wherein said enzyme uses either directly or indirectly high energy phosphate stores when catalysing a reaction so that use of said phosphate can be observed by observing the catalytic activity of the enzyme.

10. A test kit according to Claim 6 - 9 wherein said kit further comprises means for taking a blood and/or urine sample.

11. A test kit according to Claim 10 wherein said means comprises means for providing a plasma extract of said blood.

12. The use of phosphate in the manufacture of the medicament for the treatment of a fatigue related illness.

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Patents Act 1977
Examiner's report to the Comptroller under Section 17
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Relevant Technical Fields

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M R WENDT

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Databases (see below)
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE: WPI, CLAIMS, JAPIO, CAS ONLINE

Documents considered relevant following a search in respect of Claims :-
1-11

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages	Relevant to claim(s)
	NONE	

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